IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Short, et al. Art Unit : 1652
Serial No. : 09/966,803 Examiner : Delia M. Ramirez, Ph.D.
Filed : September 27, 2001
Title : ENZYMES HAVING AMIDASE ACTIVITY AND METHODS OF USE THEREOF

Commissioner for Patents
P.O. Box 1450
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DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

1. I, Jay Short, am an expert in the field of molecular biology and enzyme development and was an expert at the time of the invention. I am presently employed as CEO and as a research scientist at Diversa Corporation, San Diego, CA, assignee of the above-referenced patent application. My resume as documentation of my credentials has been submitted in a previous response.

2. I am a co-inventor on this application with Eric Mathur, Dennis Murphy, John Reid and Dan Robertson.

3. I declare that it would not have been necessary for the skilled artisan to understand which specific regions or structural elements of an amidase were necessary for function or activity to routinely generate the genus of claimed amidase-encoding nucleic acids. Methods for making and screening enzymes were sufficiently comprehensive and routine at the time of the invention to predictably generate a genus of amidase-encoding sequences without need of knowing which specific regions or structural elements of a sequence or structure affected function or activity. Methods known at the time of the invention for modifying nucleic acid and polypeptide sequences in combination with known enzyme (amidase) screening protocols (e.g., high through-put screening) made methods that required previous knowledge of structural elements necessary for enzymatic activity obsolete and unnecessary. Enzyme screening methodologies, including high through-put screening, known at the time of the invention (including *in vivo* and *in vitro* nucleic acid expression and enzyme (amidase) screening

protocols) made methods that require previous knowledge of protein structure, including secondary or tertiary structure, active site sequences, and the like obsolete and unnecessary. By using methods known in the art at the time of the invention, including the amidase screening protocols described in the specification, it would not have been necessary to understand which specific regions of amidase structure needed to be modified to generate the claimed genus of nucleic acids without undue experimentation. The specification provided sufficient guidance to one of ordinary skill in the art to make and use the genus of amidase-encoding nucleic acids to practice the methods of the invention.

4. I declare that if the skilled artisan needed guidance as to which amino acid residues could be modified to obtain structural or functional variants of an enzyme of the invention, that information was, for example, readily available in the form of amidase sequences known in the art at the time of the invention. A routine, simple sequence alignment comparison of known amidase sequences would have identified regions of identity and dissimilarity to provide guidance to the skilled artisan as to which sequences could be changed, or not changed, to generate structural and/or functional variations of amidases or enzyme-encoding nucleic acids of the invention.

5. I declare that direction to the skilled artisan as to which amino acid residues can be substituted, deleted or inserted into a nucleic acid to obtain structural, and functional, homologues of an amidase enzyme could also be found in the art at the time of the invention. For example, at the time of the invention one of skill in the art would have been aware of the many studies of amidase activity and active site structure, see, e.g., Chebrou (1996) Biochim. Biophys. Acta 1298(2):285-293, "Study of the amidase signature group"; Novo (1995) FEBS Lett. 367(3):275-279, "Pseudomonas aeruginosa aliphatic amidase is related to the nitrilase/cyanide hydratase enzyme family and Cys166 is predicted to be the active site nucleophile of the catalytic mechanism"; Tata (1994) Biochim Biophys Acta. 1205(1):139-145, "Arg-188 and Trp-144 are implicated in the binding of urea and acetamide to the active site of the amidase from Pseudomonas aeruginosa". Accordingly, one skilled in the art at the time of the invention using the teaching of the specification had many sources of direction to determine

which amino acid residues could be substituted, deleted or inserted into a nucleic acid to obtain structural, and functional, homologues of an amidase enzyme.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted

Date: JAN. 24, 2005

Jay Short